

Non-Invasive Analytical Technique for Forensic Analysis: Raman Spectroscopy

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Abstract: Forensic science is the study of sample found in traces at the scene and correlating it to identify culprit among suspects of the crime. Raman Spectroscopy is preferred choice for forensic scientist due to its reliability, versatility and other advantages over other analytical techniques. It is non-destructive, non-invasive and in-situ analytical technique which can be used as an evidence in forensic cases. It requires small amount of sample with no sample preparation, provides high degree of sample discrimination and results obtained are easy to store as a database for future references. The evidences obtained from crime scene can easily be analyzed without any alteration and diffusion of minor details. This paper discuss different types of Raman Spectroscopy and its potential in Forensic Analysis of samples.

Keywords: Raman Spectroscopy, Forensic Science, non-destructive, Crime scene, Evidence.

1. Introduction

There are various analytical techniques like spectroscopic, chromatographic, microscopic used for analysis of various forensic evidences like body fluid, fingerprints, glass, paints, inks, DNA etc. Raman spectroscopy is one of most popular analytical techniques based on Raman Scattering. It is phenomenon in which the incident photon gains or loses energy on interaction with vibrating molecules of the samples. When incident light falls on the sample, elastic and inelastic scattering occurs. In elastic scattering, there is no change in photon frequency and the process is known as Rayleigh scattering. In Inelastic scattering, there is a shift in frequency of photon due to loss or gain in energy while interaction of photon and molecules of the sample. The frequency of scattered photon will be high when incident photon gains some energy from molecules of the sample and is known as Anti-Stokes Raman Scattering. The Stokes Raman Scattering will occur when a molecule of sample gain energy from incident photon and results in lower frequency of scattered photon. Only a small fraction of light (approximately 1 in 10^7 photons) undergoes during inelastic scattering.

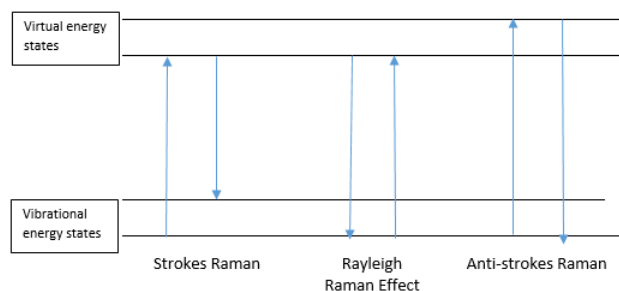


Figure 1: Raman Effect

The figure 1 shows the energy difference between scattered and incident photons. The Raman shift can be calculated through the given equation where λ wavelength in cm.

$$\nu = \frac{1}{\lambda_{\text{incident}}} - \frac{1}{\lambda_{\text{scattered}}}$$

The magnitude of Raman shift doesn't depends on wavelength of incident radiation while Raman scattering depends on wavelength. To obtain Raman spectrum, molecules should have polarizability during molecular

vibrations. As water has no polarizability so water is considered as inactive for Raman Effect and hence an ideal solvent for dissolving samples. Therefore, this technique is also useful for biological sample analysis.

As very smaller number of molecules contribute to Raman scattering, therefore the intensity of scattered light is very less. Raman spectroscopy was discovered in 1928 by Sir CV Raman, however due its very low intensity and poor sensitivity of detector, it had limited application till the invention of LASER. Raman Spectrometer is consist of light source, monochromatic, sample holder and detector. Technological advancement in Raman spectroscopy is made with use of LASER and Detector like CCD, photomultiplier tubes, FTIR and photodiode array etc. for trace level analysis. Raman spectrometer is also coupled with other analytical techniques, like high preformation liquid chromatographer, micro chromatography, scanning tunneling microscope and atomic force microscope to enhance its reliability and versatility (Mojica and Dai 2022).

Raman Spectroscopy has advantages over other analytical techniques likes.

- No sample preparation requirements.
- No interference of Spectrum by water.
- Non-destructive process.
- Immediate availability of Spectrum.
- Small amount of sample is required (1 micrometer in diameter)
- Analysis of inorganic sample

1.1 Advances in Raman Spectroscopy

1.1.1 Surface Enhanced Raman Spectroscopy (SERS)

This technique involves the enhancement of Raman signal by several order (approx. $>10^8$) for species adsorbed on rough metal surfaces compared to that obtained from same amount of molecules in solution or gas phase. The intensity of Raman signal can be enhanced by two processes: Localization of light and chemical enhancement of substrate.

The electromagnetic enhancement is due to localization of light at the surface of substrate while chemical enhancement

arises from the modification in polarizability of a molecule. This technique is capable to detect even a single molecule. In electromagnetic enhancement, nanoparticles (NP) can be developed on the substrate or deposited on substrate (Chen et al. 2006; Mojica and Dai 2022). These NP interact with the excitation source and induces the electric polarization for their surface electron. Hence surface electron oscillates in accordance with incident light and is known Surface Plasmon Oscillations. At particular frequency, the resonance between incident light and surface Plasmon takes place, and at this frequency the electric field intensity and Raman scattering from molecule of substrate which is in contact with NP are enhanced (Jones et al. 1999; Lombardi and Birke 2012).

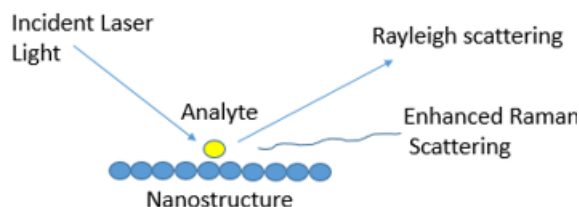


Figure 2: Surface Enhanced Raman Spectroscopy

Chemical enhancement includes enhancement of scattering due to charge transfer mechanism. The transfer of photo-induced electron from Fermi level of metal enhancement of Raman signal. Hence electromagnetic and chemical enhancement together contributes for enhancement of Raman signal to the order of 10^{14} . Thus SERS now is highly sensitive and multiplexed optical techniques for molecular analysis.

1.1.2 Coherent Anti-Stokes Raman Spectroscopy (CARS)

CARS is a technique for enhancement in the Raman signal without the involvement of any exogenous media. It is observed that molecules are irradiated using two laser beams, simultaneously operating at different frequencies, pump field at frequency ω_p and Stokes field at frequency ω_s beam interacting nonlinearly. CARS is observed when the difference between the higher frequency and lower frequency becomes equal to the vibrational frequency of the target bond molecule with the angular frequency equal to $\omega_{\text{CARS}} = 2\omega_p - \omega_s$ generating vibrational coherences. In CARS, atoms oscillate with the same phase and the atomic vibrations are coherently stimulated, leading to signal enhancement of several orders of magnitude depending on the incident power and scattered density. With the help of appropriate resonant plasmonic substrates, the sensitivity of CARS can be further increased. The same on linear mixing process was first observed by Terhune. The development in high powered & tunable pulsed laser has made possible to produce resonance phenomenon which also increases its efficiency. Therefore, this method is most suitable for high resolution Raman Spectroscopy. This technique has benefits like:-

- As net energy is not transferred to sample, so there is minimum photo-damage of sample.
- CARS is a nonlinear multi-photon technique so it provides 3D sectioning ability and video-rate imaging.
- Fluorescence doesn't interfere with signal in CARS, as anti-stokes Raman scattering (blue shifted) occurs at different frequency than fluorescence.

If the frequency of the CARS (input or output) is in resonance with the collective modes of the plasmonic nanostructure, the

CARS signals will be enhanced due to the local fields of the excited Plasmon modes. This phenomenon is called as Surface Enhanced CARS (SECARS).

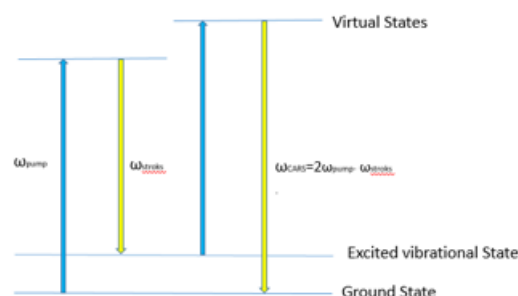


Figure 3: Coherent Anti-stokes Spectroscopy

1.1.3 Tip Enhanced Raman Spectroscopy (TERS)

It is combination of surface-enhanced Raman spectroscopy (SERS) with high spatial resolution of Scanning Probe Microscopy (SPM) and enables chemical imaging of surfaces at the nanometre length-scale. The concept of TERS was first proposed by Wessel in 1985, and it was experimentally implemented in 2000 (Mojica and Dai 2022)[2–5]. A sharp metal or metal coated SPM tip is positioned at the center of a laser focus and the electromagnetic field (EM) at the tip apex is confined and enhanced due to the combination of the localised surface Plasmon (LSP) resonance and lightning rod effect. This increase of the electromagnetic field enhances the Raman signal from the molecules in the vicinity of the tip-apex and enables nanoscale chemical imaging of a surface, overcoming the diffraction limit of SERS and conventional Raman spectroscopy.

The tip acts as a nano-source of light and local field enhancer, greatly improving the Raman sensitivity (by a factor of $10^3 \cdot 10^7$) and reducing the probed volume to the “nano” region immediately below the tip. This increase in EM field enhances the Raman signal from the molecules in the vicinity of the tip-apex and enables nanoscale chemical imaging of a surface, overcoming the diffraction limit of SERS and conventional Raman spectroscopy. Hence TERS has extended the spatial resolution to nanoscale level.

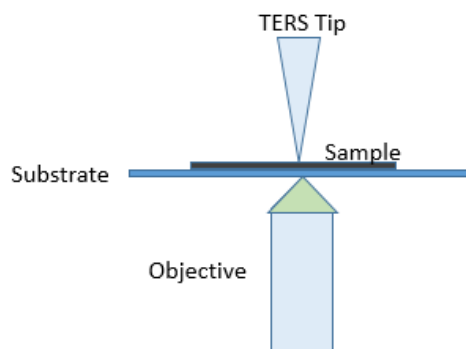


Figure 4: Tip Enhanced Raman Spectroscopy

1.1.4 Resonance Hyper Raman Spectroscopy (RHRS)

It is modified form of Raman Scattering in which scattering of light occur at optical frequencies lower or equal to twice the frequency of pump light and give rise to Hyper and Resonance Hyper Raman Spectroscopy. Hence, two pump photons are converted into one photon of Raman scattered light and one phonon. It is modified version of Raman Scattering and

scattering light occurs at optical frequencies lower than twice the frequency of pumping light. Hence two pumping photons get converted into one photon of Raman scattered light and one phonon.

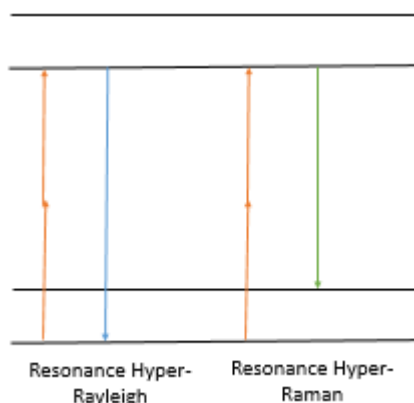


Figure 5: Resonance Raman Spectroscopy

1.1.5 Confocal Raman Spectroscopy (CRS)

It brings confocal imaging by coupling microscope and Raman spectroscopy to enhance resolution. The sample is placed on microscope and laser light is used as excitation source. Rayleigh and Raman scattered light is collected by objective lens, transmitted through beam splitter and pass-through filter to remove Rayleigh light. Raman scattered light is used for studying wavelength and spectrum (Baran et al. 2023). 3D sample information can be obtained by moving microscope stage. CRS is powerful techniques to perform identification and mapping of multilayer samples minerals, particulates identification and chemical distribution.

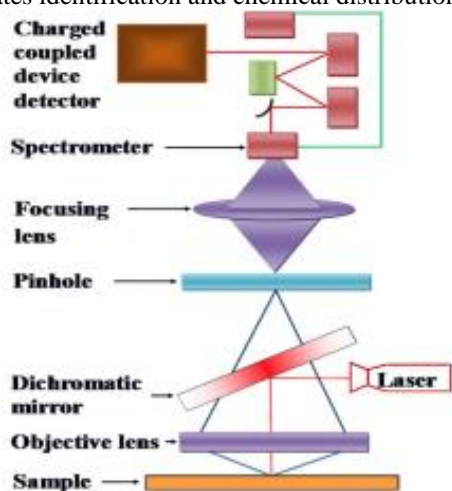


Figure 6: Confocal Raman Spectroscopy

1.1.6 Energy Dispersive Raman Spectroscopy (EDRS)

This is one of the powerful technique for elemental analysis. The basic principle is the interaction of high energy electromagnetic radiation and sample which will result in ejection of core electron. Ejected electron creates a hole which will be filled by an electron from higher energy. Based on Mosely Law, atomic number and quantity of sample can be determined. This is non-destructive and is usually coupled with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to study insight into structure and morphology of sample.

1.1.7 Stimulated Raman Spectroscopy (SRS)

It is non-linear optical technique in which two coherent polarized lights are used at excitation source. The stimulated Raman spectroscopy occurs when the difference of incident pump (ω_{pump}) and stroke pump frequency matches particularly with the molecular vibrational frequency. The stimulated Raman signal can be measured by either increase of power in strokes laser which is called stimulated Raman gain (SRG) or decrease of power in pump laser which is stimulated Raman loss (SRL). This technique provides non-resonant background free and better contrast image.

1.1.8 Spatially Offset Raman Spectroscopy (SORS)

Most of Raman Spectroscopy depends on backscattering phenomena and gives more information about surface and less about subsurface. Spatially offset Raman spectroscopy is study the subsurface information from diffusely scattered media and is mainly used in forensic investigation. There is much probability that the photon get inelastic scattered and exit at an offset from illumination spot on the surface. Thus Raman signals collected by probe at different offset distance gives the information about its subsurface. Hence, it is depth profiling, non-invasive technique to study chemical identification and composition of a material present under the surface up to few millimeters. This technique is mainly used in screening, drug detection, counterfeit products or illicit substance and forensic testing of evidences without altering it.

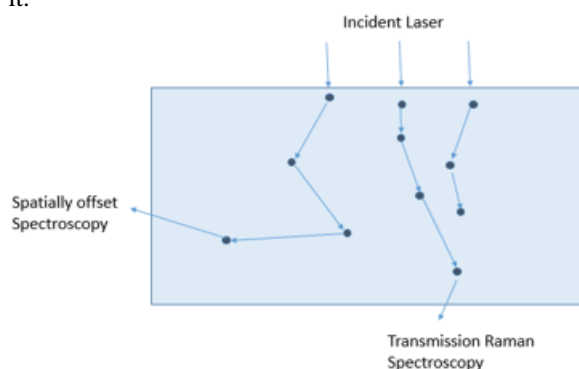


Figure 7: Spatially Offset Raman Spectroscopy

1.1.9 Shifted Excitation Raman Difference Spectroscopy (SERDS)

This Raman spectroscopy uses the background correction method to reduce the auto-fluorescence effect directly through surface packaging and container materials for sensitive forensic evidence. This removal of fluorescence background is based on Kasha-Vavlov rule. It states that the luminescence phenomena occurs due to relaxation of molecule from lowest excitation state to the ground state. Molecules can be excited to different energy levels in an excited state by different excitation wavelengths. These excited molecules first under goes non radiative relaxation to lowest excited state before jumping to the ground state. The excitation wavelength should be chosen in such a manner that shifted Raman spectra should have same fluorescence profile since same fluorophores are excited. The subtraction of shifted Raman spectra from each other will provide Raman information without any background. In order to get fluorescence free and enhanced Raman spectrum, SERDS is combined with SERS and is known as surface- enhanced shifted excitation Raman difference spectroscopy (SESERDS)

1.1.10 Transmission Raman Spectroscopy (TRS)

Transmission Raman spectroscopy can be considered as one of special case of spatially offset Raman spectroscopy. This technology is insensitive to surface, requires no sample preparation, and rapid process which is used to probe opaque or turbid materials. This technique is mostly used in analyzing the bulk content of diffusely scattered material and give information about physical state, qualitative and quantitative information.

1.2. Application of Raman Spectroscopy in Forensic Science

Forensic science is greatly benefitted from Raman spectroscopy than any other analytical technique. Raman spectroscopy is non-destructive, non-invasive, eco-friendly, robust, fast acquisition, extraction free, portable, multi-compound detection technique widely used in forensic science for the detection and characterization of samples. As Raman spectroscopy is a non-destructive technique, highly sensitive and requires no sample preparation, thus sample can be considered as evidence in a court case. Raman spectroscopy requires a small amount of sample for analysis like traces of evidence left at crime spot. Hence, Raman spectroscopy is vital and extremely essential for forensic analysis. Courts always prefer to preserve evidences in their original state which is very much possible while employing Raman spectroscopy for forensic analysis.

1.2.1 Blood and body fluids

Blood and body fluid samples play significant role in forensic investigation. Studies of Raman scattering of human blood identities includes the donor as well as its age and dilution. Other bodily fluids such as tears, saliva, semen, urine, vaginal secretion and sweat can also be tested. A minuscule amount of sample can also provide a lead in predicting the culprit if examined carefully. Raman spectroscopy shows less interference from water like other techniques and hence it is great techniques for analysing body fluid and their traces. Nowadays, portable Raman spectrometer along with advanced software is available in situ for accurate analysis at crime scene. Fresh blood exhibits the peak at 1155 and 1511 cm^{-1} (Boyd, Bertino, and Seashols 2011) which disappeared upon drying and it can be used to determine the age of blood. There is also reduction in oxy-hemoglobin peak in dry blood than that dry blood.

1.2.2 Analysis of ink samples on questioned documents

Questioned documents are suspicious documents and need forensic examination. It can be handwriting, signature, computerized, typewritten/ printed document whose reliability is doubtful. Ink and paints consist of complex homogenous structure. Multiple analytical technique are required for physical, chemical and structural characterizes for complete study. The sample preservation is priority in forensic science and hence, it is often not ideal to conduct analytical chemical testing on questioned document. Raman spectroscopy offers the advantage to chemically analyses the sample without the preparation and without losing the evidential value of document. Paper exhibit fluorescence and hence dominates over the Raman signal. Also the synthetic dye used in textiles, ink and paints shows fluorescence effect and heating effect any lead to alteration or damage of

questioned documents. Therefore concave baseline correction method is used to eliminate the effect of fluorescence superimposed on Raman spectrum [2]. The intensity of Raman spectrum can also be enhance by surface enhance Raman scattering. A recent study shoes that organic colorants presents in inks, paints and textile fibers cab be easily identified from micro-sample by treatment of the sample with the silver nanoparticles before analysis (Leona et al. 2011). In dye based specimens, surface enhanced Raman scattering (SERS) is used to overcome fluorescence phenomena (Claybourn and Ansell 2000; Fleischmann, Hendra, and McQuillan 1974). In case of any document alteration or overwriting, confocal Raman spectroscopy is used to study depth resolution.

1.2.3 Fibre and Physical evidence

Textile fibre are most dominate evidence, when analyzed properly, the link between suspect, victim and crime scene can be created. Raman spectroscopy is also used in forensic examination of textile fibre to identify the generic fibre class and sub class, comparing the fibre trace with controlled sample and also in identification of type of dye use in fibre. In general dispersive Raman instrument coupled with high resolution microscope is used. To reduce fluorescence impact, NIR laser source can be used. To overcome the florescence effect, baseline correction and surface-enhanced Raman scattering (SERS) is also used in forensic lab to test physical evidences collected from crime scene. The natural fibre like wool, cotton and silk are weak Raman scatters than man-made fibres. The nylon subclasses Raman peak will be found around 940 cm^{-1} (De Wael and Lepot, 2017).

The Raman spectrum shows intense band for dye or pigment and medium peaks (polyester), weak peaks (acrylic and nylon) or sometimes no peaks (cellulose) from the fibre. The subtraction of polymer peaks gives the detailed information about the dye used. Thus this technique offers the unique methodology to identify the chemical composition of fibre, its subclass and also dyestuffs used.

1.2.4 Gunshot Residue

In most of criminal cases, Gunshot residue (GSR) can be crucial evidence in criminal cases when no weapon is found on crime scene. The identification of evidences and their investigation will help in correlating the suspect with the crime. GSR are debris obtained from firearm and is mixture of burnt and unburnt and organic and inorganic material. The burning rate and power generated depends on size and shape of propellant used. FTIR with Raman spectroscopy provides powerful spectral signature and help in differentiation of different gunpowder bases on their particle size and composition. The presence of heavy metal particles left by cartridge primer is base for GSR detection in any analytical techniques. It can be analyzed with the help of scanning electron microscopes with energy dispersive spectroscopy (SEM/EDS). However the introduction of heavy metal free or toxic free ammunition leads to problem of identification of it. Now days, Raman Spectroscopy is used for organic analysis of GSR. Even minute trace can be identified without altering any evidence by Raman scattering.

1.2.5 Illicit drugs

The surface enhanced Raman spectroscopy and surface enhanced resonance Raman spectroscopy is widely used for trace forensic analysis and classification of active pharmaceutical ingredient and counterfeit drug samples (Buckley and Ryder 2017). Nowadays Raman technology is also widely used in pharmaceutical industry for the different purpose like quantitative and qualitative analysis in product formation, identification of raw materials, polymorphism screening and identification etc. (Koutrakos, Leary, and Kammrath 2018). These techniques are well efficient to detect the trace of sample in biological sample (blood, urine or in other diluted form). Illicit drugs are highly addictive, abuse and dangerous e.g. cocaine, hallucinogens, marijuana etc. The spectral database of the all the material used in processing will provide convenient and robust method of identify the contamination (Wang et al. 2018; West and Went 2011). Various branches of Raman spectroscopy is used in forensic analysis due to its non-destructive nature, less time consuming and non-contact.

2. Conclusion

It is very crucial to connect the crime sequence and identify suspect while preserving evidence. Scientific study of these samples should include non-invasive, non-destructive and no sample preparation techniques. Raman spectroscopy is one of non-destructive, non-invasive (through glass, plastic or turbid media) able to probe in aqueous solution, and even for trace sample. The biggest drawback associate with it is Raman scattering process and fluorescence interference which limits the sensitivity of the technique. The advancement in Raman technology has increased its interference in accurate and effective evidence analysis. It is emerged as one of the best analytical tools, as it withstands required evidence analysis without any alteration of the sample and preserve the same for future references & testing.

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